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Human myeloma cells promote the production of interleukin 6 by primary human osteoblasts

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Summary. Interleukin-6 (IL-6) is an important growth and survival factor for myeloma cells. However, the identity of the cells producing IL-6 *in vivo* remains unclear. Myeloma cells are found closely associated with sites of active bone turnover, and cells of the osteogenic lineage, including bone marrow osteoprogenitors, osteoblasts and bone lining cells, may therefore be ideally placed to synthesize IL-6. We have examined the possibility that human osteogenic cells may produce IL-6 in response to stimulation by myeloma cells. Primary human osteoblasts (hOBs) were isolated from normal donors, co-cultured with the human myeloma cell lines, JJN-3, RPMI-8226 and NCI-H929, and the amount of IL-6 released was determined by enzyme-linked immunosorbent assay (ELISA). All myeloma cells stimulated a significant increase in the production of IL-6 when cultured with hOBs ($P < 0{\cdot}05$). Prior fixation of hOBs completely abrogated release of IL-6 in the co-cultures. In contrast, fixed myeloma cells retained the ability to induce IL-6 production, suggesting that hOBs were the principal source of IL-6. Physical separation of myeloma cells from hOBs using transwell inserts caused a partial inhibition of IL-6 release ($P < 0{\cdot}05$), whereas the addition of media conditioned by myeloma cells to cultures of hOBs stimulated a significant increase in IL-6 production ($P < 0{\cdot}05$). hOBs secreted greater amounts of IL-6 than human bone marrow stromal cells (hBMSCs) (2·2- to 3·5-fold, $P < 0{\cdot}05$), but incubating hBMSCs with dexamethasone to stimulate osteoblastic differentiation resulted in an increase in their ability to produce IL-6 (1·7- to 4·8-fold, $P < 0{\cdot}05$) and to respond to myeloma cells ($P < 0{\cdot}05$). These data clearly indicate that cells of the osteoblast lineage release significant amounts of IL-6 in response to stimulation by myeloma cells and may contribute to the IL-6 that promotes the proliferation and survival of myeloma cells *in vivo*.

Keywords: multiple myeloma, osteoblast, interleukin-6, bone marrow, dexamethasone.

Multiple myeloma (MM) is a differentiated B-cell neoplasm characterized by the clonal proliferation of plasma cells that accumulate in the bone marrow. Patients with multiple myeloma commonly develop characteristic osteolytic bone disease, which has proved to be a major cause of morbidity and mortality. Recent studies have suggested that there may be considerable interdependence between myeloma and bone, with myeloma cells producing factors that promote bone resorption and the cells of bone, including osteoclasts, producing factors that may be important in the proliferation and/or survival of myeloma cells (Garrett *et al.*, 1995; Bataille *et al.*, 1996). A number of local growth factors, including interleukin-1β (IL-1β) (Cozzolino *et al.*, 1989; Kawano *et al.*, 1989), lymphotoxin (Garrett *et al.*, 1987; Bataille *et al.*, 1989a), interleukin-6 (IL-6) (Kawano *et al.*, 1988; Klein *et al.*, 1989) and the soluble receptor for IL-6 (Gaillard *et al.*, 1993; Tamura *et al.*, 1993), have been implicated in contributing to this relationship. However, the precise role of each of these factors *in vivo* remains unclear.

IL-6 has been shown to play a major role in the pathophysiology of MM, as it is an important growth factor for malignant plasma cells both *in vitro* and *in vivo* (Kawano *et al.*, 1988; Anderson *et al.*, 1989; Bataille *et al.*, 1989b; Klein *et al.*, 1989, 1991; Nilsson *et al.*, 1990). Recent reports have also suggested that IL-6 may be an important survival factor for myeloma cells, as IL-6 can prevent

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dexamethasone-induced apoptosis *in vitro* (Hardin *et al*., 1994; Lichtenstein *et al*., 1995). Furthermore, a number of studies have suggested that IL-6 may play an important role in stimulating bone resorption through increased recruitment and activation of osteoclasts (Kurihara *et al*., 1991; Roodman, 1992; Tamura *et al*., 1993). However, the identity of the cells responsible for producing IL-6 *in vivo* remains controversial. Kawano *et al*. (1988) first postulated that IL-6 was produced in an autocrine manner, as partially purified (95%) myeloma cells were shown to synthesize IL-6. This hypothesis was supported by studies demonstrating that myeloma cells express the mRNA for IL-6 and the IL-6 receptor (Anderson *et al*., 1989; Freeman *et al*., 1989; Sati *et al*., 1998). Furthermore, myeloma cell lines have been described that produce and respond to IL-6, the growth of which can be inhibited by antibodies to IL-6 and antisense oligonucleotides (Levy *et al*., 1991; Schwab *et al*., 1991). Moreover, myeloma cells have been shown to express CD40, which can be triggered by CD40 ligand to produce IL-6 (Westendorf *et al*., 1994; Urashima *et al*., 1995). In contrast, other studies favour a paracrine production of IL-6, as only a small proportion of myeloma cells were shown to secrete IL-6 (Hata *et al*., 1993; Klein *et al*., 1995). Stromal cells have also been shown to be a major source of IL-6, and myeloma cells can stimulate IL-6 secretion from these cells in a contact-mediated manner (Klein *et al*., 1989; Caligaris Cappio *et al*., 1991; Uchiyama *et al*., 1993; Lokhorst *et al*., 1994).

Given the close association between myeloma cells and bone, it is likely that additional cells, including osteoclasts and osteoblasts and/or bone lining cells, may also be important in producing IL-6 in response to interactions with myeloma cells. In support of this, Garrett *et al*. (1995) have demonstrated that osteoclasts are an important source of IL-6 and that IL-1β and lymphotoxin can promote the release of IL-6 from isolated osteoclasts *in vitro.* Furthermore, Barille *et al*. (1995) have demonstrated that an IL-6-dependent myeloma cell line can induce IL-6 production from osteoblastic osteosarcoma cells. Therefore, the aim of the present study was to determine whether primary human osteoblasts synthesize IL-6 in response to interactions with human myeloma cells. Furthermore, the relative ability of primary osteoblasts and human bone marrow stromal cells to produce IL-6 in response to myeloma cells was also determined.

MATERIALS AND METHODS

Materials. RPMI-1640 medium, minimum essential medium (MEM), fetal bovine serum (FBS), Dulbecco's phosphate-buffered saline (PBS), L-glutamine, trypsin/EDTA, penicillin and streptomycin, MEM non-essential amino acids, sodium pyruvate and 96-well Nunc immunoplates were obtained from Gibco Life Technologies (Paisley, UK). Transwell inserts and all other tissue culture plastics were purchased from Costar (High Wycombe, Bucks, UK). Rabbit polyclonal anti-human IL-6 antibody and biotinylated rabbit polyclonal anti-human IL-6 antibody were obtained from Professor Klaus Bendtzen (Institute for Inflammation Research, Tagensvej, Copenhagen, Denmark). Recombinant human IL-6 (specific activity of 2×10^7 U/mg) was a gift from Sandoz Pharma (Basle, Switzerland). Paraformaldehyde, human serum albumin, streptavidin–peroxidase, 1,25 o-phenylenediamine dihydrochloride, histopaque 1077, trypan blue, mitomycin C, 2-mercaptoethanol and dexamethasone were purchased from Sigma Chemicals (Poole, Dorset, UK). All other chemicals were from BDH Chemicals (Poole, Dorset, UK).

Culture of human myeloma cell lines. The human myeloma cell lines, RPMI-8226 and NCI-H929, were obtained from the European Tissue Culture Collection (Porton Down, UK). The human myeloma cell line, JJN-3, was kindly provided by Professor I. Franklin (University of Glasgow, UK). All cells were grown in RPMI-1640 medium supplemented with 10% FBS, 2 mmol/l L-glutamine, 5 μmol/l 2-mercaptoethanol, 2 mmol/l sodium pyruvate and 0·1 mmol/l MEM non-essential amino acids in a humidified atmosphere of 5% CO_2 in air at 37°C, unless otherwise stated. Medium conditioned by myeloma cells was prepared from cells plated at a density of 1×10^6 cells/ml for 48 h. Supernatants were collected, filtered (0·45 μm acrodisk; Gelman Sciences, Northampton, UK) and stored at −20°C until assayed.

Preparation of primary human osteoblasts. Primary human osteoblasts (hOBs) were obtained from human trabecular bone explants, using a modification of the method described by Beresford *et al*. (1983, 1984). hOBs were allowed to grow out from explants until confluent (usually 4–6 weeks) and then processed appropriately. These cells have been characterized extensively and shown to express the major characteristics of osteoblasts (Beresford *et al*., 1983, 1994; Skjodt *et al*., 1990; Gallaghar *et al*., 1996). Cells were cultured in MEM supplemented with FBS (10% v/v), L-glutamine (2 mmol/l), penicillin (100 IU/ml) and streptomycin (100 μg/ml).

Preparation of human bone marrow stromal cells. Human bone marrow stromal cells (hBMSCs) were isolated and cultured according to the method of Cheng *et al*. (1994). Human bone marrow specimens were obtained from either ribs or femoral heads excised during surgery or by aspiration from the posterior iliac crest of normal healthy donors. Mononuclear cells were isolated by density gradient centrifugation, placed into culture flasks at a density of 5×10^4 cells/cm^2 and incubated for 7 days to allow attachment in MEM supplemented with FBS (10% v/v), L-glutamine (2 mmol/l), penicillin (100 IU/ml) and streptomycin (100 μg/ml). After this period, half of the culture medium was replaced with fresh medium and, thereafter, the cells were fed twice a week. The cells were allowed to grow to confluence, usually 18–21 days, and processed appropriately. In certain experiments, hBMSCs were incubated in the presence of dexamethasone (10^{-8} mol/l) for the final 7–10 days of the culture period to induce osteogenic differentiation (Beresford *et al*., 1994; Cheng *et al*., 1994). These cells were then processed appropriately (see next section). Dexamethasone was therefore not present when cells were cultured with myeloma cells or when IL-6 concentrations were being determined.

Co-cultures of human myeloma cells with primary osteoblasts or bone marrow stromal cells. Confluent monolayer cultures of either hOBs or hBMSCs were treated with mitomycin C (1 μg/ml) for 3 h to inhibit mitosis and then washed three times with sterile PBS. The cells were then harvested by trypsinization [trypsin (5% w/v)/EDTA (2% w/v)], washed and seeded into 24-well plates at a density of 5×10^4 cells/cm^2. After incubation for 24 h, the culture supernatants were removed and the cells washed once with serum-free RPMI-1640. Human myeloma cells were seeded onto the monolayers as described below.

Human myeloma cells were incubated with mitomycin C (1 μg/ml) for 3 h, washed and then resuspended in RPMI-1640 medium. The cells were adjusted to a density of 1×10^6 cells/ml, and 1 ml of cell suspension was added to each well of a 24-well culture plate containing hOBs or hBMSCs. After incubation for 48 h, the supernatants from these co-cultures were collected, centrifuged to remove cells and stored at −20°C.

In experiments designed to determine the identity of cells producing IL-6 in the co-culture system, the different components of the system were mildly fixed by incubating the cells with 1% paraformaldehyde for 10 min. The cells were then washed extensively with PBS and established in co-culture. Experiments were also performed to determine whether soluble factors mediated IL-6 production or whether cell-to-cell contact was important. Thus, instead of myeloma cells, media conditioned by myeloma cells (10% v/v) were added to cultures of human osteoblasts. Alternatively, co-cultures were established in which the two cellular components were separated by transwell inserts, which allow the passage of soluble factors but prevent physical contact. After incubation for 48 h, the supernatants were removed, centrifuged to remove contaminating cells and stored at −20°C until IL-6 concentrations could be measured.

Measurement of IL-6 using ELISA. IL-6 was measured by an enzyme-linked immunosorbent assay (ELISA) using a modification of the method described by Hansen *et al.* (1991). Briefly, 96-well Nunc immunoplates were coated overnight at 4°C with rabbit polyclonal anti-human IL-6 antibody (4 μg/ml) in coating buffer (40 mmol/l Na_2HPO_4, 6 mmol/l $NaH_2PO_4 \cdot 2H_2O$ and 100 mmol/l NaCl, pH 7·4). Unbound antibody was removed, and the plates were blocked overnight at 37°C with 1% human serum albumin (HSA) in PBS. IL-6 standards diluted in incubation buffer (coating buffer, 0·5% HSA, 0·15% Tween 20) or the appropriate culture supernatants were added, and the plates were incubated overnight at 4°C. The plates were then washed and incubated with biotinylated rabbit polyclonal anti-human IL-6 antibody (4 μg/ml), diluted in incubation buffer, for 2 h at room temperature. The plates were incubated with streptavidin–peroxidase (0·5 μg/ml) for 1 h at room temperature. After further washing, 1,25 o-phenylenediamine dihydrochloride (0·067 mg/ml) diluted in 0·1 mol/l citrate-phosphate buffer and H_2O_2 (0·015%) were added, and the colour was allowed to develop. The reaction was terminated by the addition of 2·5 mol/l H_2SO_4 before reading the absorbance at a wavelength of 492 nm. A standard curve was established, and the concentration of IL-6 in each sample was determined by interpolation. The lower limit of detection in the ELISA was 20–30 pg/ml.

Measurement of alkaline phosphatase. hBMSCs were seeded in 12-well plates at a density of 1×10^5 cells/well in MEM supplemented with FBS (10% v/v), L-glutamine (2 mmol/l), penicillin (100 IU/ml), streptomycin (100 μg/ml), ascorbic acid (50 μg/ml), 1,25-dihydroxy vitamin D_3 (10^{-8} mol/l) and vitamin K_1 (10^{-8} mol/l) in the presence or absence of dexamethasone (10^{-8} mol/l) or vehicle. After incubation for 72 h, alkaline phosphatase activity was determined by hydrolysis of *para*-nitrophenyl phosphate (Phosphatase alkaline kit; BioMerieux, Marcy I'Etoile, France) (Fromigue

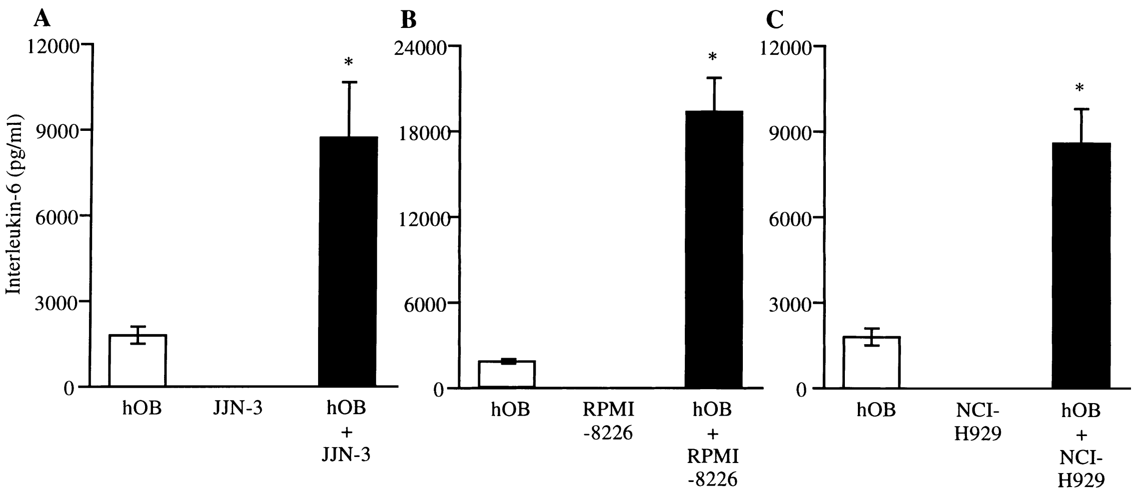

Fig 1. IL-6 production in the co-culture of primary human osteoblasts (hOBs) with JJN-3, RPMI-8226 or NCI-H929 human myeloma cells. hOBs and human myeloma cells were cultured either alone or together, and the concentration of IL-6 in the supernatants was measured using ELISA. Data represent the means ± SD of four replicates from one of five experiments. Statistical analyses were performed using the Mann–Whitney *U*-test. *$P < 0·05$ compared with IL-6 production by hOBs cultured alone.

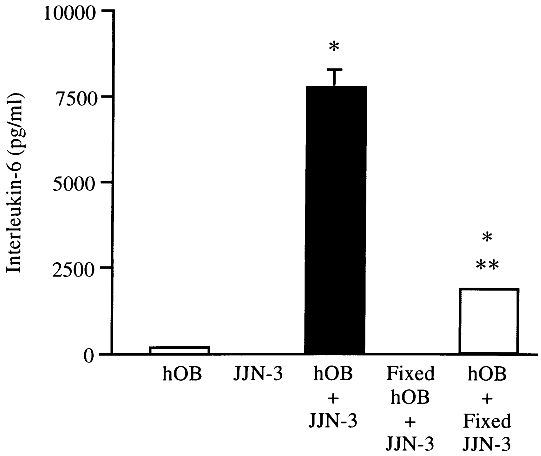

Fig 2. Identification of the cells responsible for the production of IL-6 in the myeloma/hOB co-culture system. Fixed or unfixed hOBs were co-cultured with fixed or unfixed myeloma cells, supernatants were collected, and the concentration of IL-6 was determined using ELISA. The data shown are the means ± SD of four replicates from one of two experiments. The Mann–Whitney *U*-test was performed for statistical analysis. $*P<0{\cdot}05$ compared with the amount of IL-6 measured in cultures of hOBs. $**P<0{\cdot}05$ compared with the amount of IL-6 measured in the co-culture of JJN-3 cells and hOBs.

et al, 1997; de Pollak *et al*, 1997). The total protein content within lysates was determined using a Bio-Rad protein assay (Bio-Rad, Ivry sur Seine, France). Results were expressed as nmol of *para*-nitrophenol released per min/mg protein.

Statistical analysis. The Mann–Whitney *U*-test for unpaired data was used to make comparisons between samples. The concentrations of IL-6 are expressed as the means ± SD of four replicates. All experiments were repeated on a minimum of two separate occasions. In each case, the data from a single representative experiment are shown. A 95% level of significance was accepted as minimum.

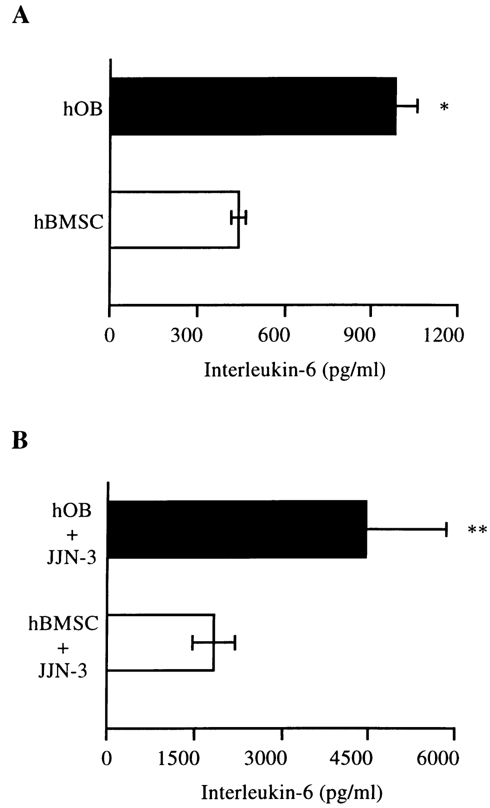

Fig 3. hOBs release greater concentrations of IL-6 than hBMSCs. hOBs and hBMSCs were isolated from the same donor and cultured alone (A) or with JJN-3 human myeloma cells (B) for 48 h. Supernatants were collected, and IL-6 concentration was assessed by ELISA. Data shown are the means ± SD of four replicates from one of three experiments. Statistical analyses were performed using the Mann–Whitney *U*-test. $*P<0{\cdot}05$ compared with the amount of IL-6 produced by hBMSCs. $**P<0{\cdot}05$ compared with the amount of IL-6 produced by hBMSCs when cultured with JJN-3 myeloma cells.

Table I. Cell-to-cell contact and soluble mediators contribute to the production of IL-6 by hOBs.[a]

	IL-6 concentration (pg/ml)		
Culture conditions	JJN-3	RPMI-8226	NCI-H929
hOBs alone	4631·5 ± 225·1	4631·5 ± 225·1	4631·5 ± 225·1
hOBs + myeloma cells	6743·0 ± 650·9*	8087·5 ± 302·7*	6437·3 ± 250·3*
hOBs + myeloma cells without contact[b]	5887·8 ± 427·9*	6333·1 ± 206·4*†	4598·9 ± 601·1†
hOBs + MCCM[c]	5891·4 ± 383·5†	7186·9 ± 138·0*†	5201·5 ± 328·0*†

[a] Data are the means ± SD of four replicates from one of two identical experiments.

[b] Physical contact between myeloma cells and osteoblasts was prevented by separating the cells using transwell inserts.

[c] Myeloma cell-conditioned media.

$*P<0{\cdot}05$ compared with hOBs cultured alone.

$\dagger P<0{\cdot}05$ compared with myeloma cells co-cultured with hOBs.

RESULTS

Human myeloma cells induce the synthesis of IL-6 in culture with human osteoblasts

hOBs were shown constitutively to produce IL-6 (432·6 ± 43·6 to 4776·9 ± 363·7 pg/ml), although there was considerable donor-to-donor variability. In contrast, IL-6 was not detected in the supernatants of the three human myeloma cell lines examined. However, when each of these myeloma cell lines was maintained in co-culture with hOBs, there was a significant increase in IL-6 production (Fig 1). Co-culturing RPMI-8226 or JJN-3 cells with hOBs increased the concentration of IL-6 released into the co-culture supernatant by 1·9- to 11·3-fold ($P < 0{\cdot}05$) and by 1·5- to 11·3-fold ($P < 0{\cdot}05$) respectively. NCI-H929 cells also increased IL-6 production in the co-culture (1·4- to 5·3-fold, $P < 0{\cdot}05$); however, the magnitude of the response was not as great as that seen with JJN-3 or RPMI-8226 cells.

hOBs are the cells responsible for IL-6 production in the co-culture system

In order to identify the cells responsible for the increased IL-6 production in the co-cultures, experiments were performed in which the osteogenic cells or myeloma cells were fixed with 1% paraformaldehyde for 10 min before co-culture. Fixation of hOBs in the co-culture system completely inhibited IL-6 production (Fig 2). In contrast, prior fixation of the myeloma cells did not inhibit IL-6 production (Fig 2), although the amounts of IL-6 released were significantly decreased when compared with cultures in which neither cell type had been fixed (Fig 2).

Cell-to-cell contact and soluble factors both contribute to the production of IL-6 by hOBs in co-culture with myeloma cells

To determine whether contact between myeloma cells and hOBs was essential to promote the production of IL-6 by the latter cell type, co-cultures were established with transwell inserts, which prevent cell-to-cell contact but allow the exchange of soluble factors. hOBs were also cultured with media conditioned by myeloma cells in order to examine independently the effect of soluble factors released by myeloma cells. All the human myeloma cell lines examined stimulated the release of IL-6 from hOBs in the experiments with transwell inserts (Table I). In the absence of physical contact, JJN-3 and RPMI-8226 myeloma cells remained able to stimulate an increase in production of IL-6 by hOBs

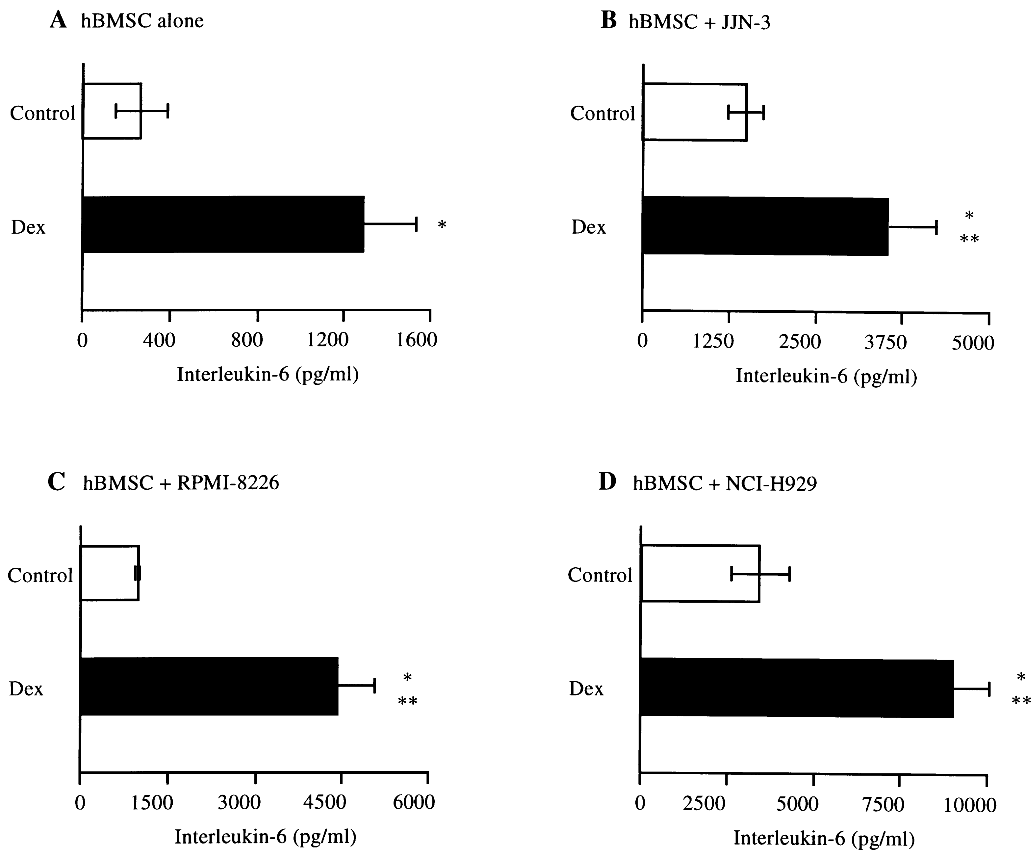

Fig 4. Dexamethasone promotes an increase in the release of IL-6 from hBMSCs. hBMSCs were cultured in the presence or absence of dexamethasone and then incubated either alone (A) or with JJN-3 (B), RPMI-8226 (C) or NCI-H929 (D) human myeloma cells for 48 h. Supernatants were collected, and the concentration of IL-6 in the supernatants was assessed by ELISA. Data shown are the means ± SD of four replicates from one of three experiments. Statistical analyses were performed using the Mann–Whitney *U*-test. *$P < 0{\cdot}05$ compared with the respective controls. **$P < 0{\cdot}05$ compared with hBMSCs cultured alone in the presence of dexamethasone.

($P<0{\cdot}05$ in each case) (Table I). Media conditioned by each of the myeloma cell lines were also able to stimulate a significant increase in IL-6 production from hOBs ($P<0{\cdot}05$ in each case) (Table I), although the magnitude of this increase was generally lower than that observed in the respective co-cultures.

Myeloma cells stimulate a greater production of IL-6 from hOBs than from hBMSCs

Although both hOBs and hBMSCs produced high levels of IL-6 when co-cultured with human myeloma cells (Fig 3), the amount of IL-6 produced varied between donors. To investigate the relative ability of either cell type to produce IL-6 in response to stimulation by myeloma cells, cultures of hOBs and hBMSCs were established from the same donor and incubated with myeloma cells. When hOBs and hBMSCs were cultured alone, hOBs released 2·2- to 3·5-fold more IL-6 than hBMSCs ($P<0{\cdot}05$) (Fig 3A). When JJN-3 myeloma cells were cultured with hOBs, there was a significantly greater induction of IL-6 than when cultured with hBMSCs (1·4- to 2·4-fold, $P<0{\cdot}05$) (Fig 3B).

Cells of the osteogenic lineage contribute significantly to the IL-6 produced by hBMSCs

hBMSC cultures are a heterogeneous population of cells containing a number of cell types, including cells of the osteoblast lineage. As hOBs produce greater amounts of IL-6 than hBMSCs, the relative proportion of cells of the osteogenic lineage within these cultures may account for this difference. To investigate this, hBMSCs were treated with dexamethasone (10^{-8} mol/l) to promote their differentiation into cells with a mature osteoblast phenotype (Cheng *et al*., 1994). Treatment of hBMSCs resulted in a significant increase in alkaline phosphatase expression in these cells (3·8-fold, $P<0{\cdot}05$). hBMSCs treated with dexamethasone produced significantly greater amounts of IL-6 than untreated cells (1·7- to 4·8-fold, $P<0{\cdot}05$) (Fig 4A). Interestingly, all three myeloma cell lines stimulated an increase in IL-6 production from dexamethasone-treated hBMSCs ($P<0{\cdot}05$), and this was significantly greater than that seen in the co-cultures of myeloma cells and untreated hBMSCs ($P<0{\cdot}05$) (Fig 4B–D).

DISCUSSION

In the present study, we have shown that human myeloma cells stimulate an increase in the production of IL-6 when co-cultured with hOBs. Although myeloma cells did not secrete IL-6 constitutively, it was possible that contact between these cells and hOBs induced IL-6 production by the myeloma cells. However, this possibility was excluded by the demonstration that mild fixation of hOBs, before co-culture, completely inhibited IL-6 production, whereas fixation of human myeloma cells was unable to inhibit synthesis of IL-6, indicating that osteoblasts are the cells responsible for producing IL-6 in this system. Physical separation of myeloma cells and osteoblasts with transwell inserts indicated that direct cell-to-cell contact contributed significantly to the induction of IL-6.

These data are consistent with previous reports demonstrating that myeloma cells can promote the production of IL-6 from hBMSCs in a contact-dependent manner (Caligaris Cappio *et al*., 1991; Uchiyama *et al*., 1993; Lokhorst *et al*., 1994). Interestingly, Tanaka *et al*. (1995) reported that the adhesion of T cells to human primary osteoblasts induced the synthesis of IL-6 in the osteoblasts. More importantly, the data described in the present study are supported by observations reported previously by Barille *et al*. (1995), who demonstrated that the IL-6- dependent human myeloma cell line, XG1, could induce IL-6 production by SaOS-2 or MG-63 human osteoblastic osteosarcoma cells. Interestingly, we were also able to demonstrate that several other human myeloma cell lines, including JJN-3 and U-266, were able to stimulate the production of IL-6 by SaOS-2 cells and MG-63 (data not shown).

In addition to direct cell-to-cell contact mediating the increased production of IL-6, media conditioned by human myeloma cells also stimulated an increase in the production of IL-6 by hOBs. Media conditioned by all three cell lines examined elicited a strong response in hOBs, with similar levels of IL-6 being released to those observed in the co-culture system. Interestingly, although Barille *et al*. (1995) concluded that cell-to-cell contact was required for optimum IL-6 production, their data may also suggest a role for a soluble mediator of IL-6 production. In experiments using transwell inserts to prevent contact between myeloma cells and SaOS-2 osteosarcoma cells, IL-6 concentrations were increased in one experiment, marginally increased in another and unchanged in the final experiment (Barille *et al*., 1995). Taken together, these data suggest that, in addition to direct cell-to-cell contact, soluble factors may contribute to the regulation of IL-6 production by human osteoblasts.

Although the identity of the factor(s) in the media conditioned by myeloma cells that may be responsible for the induction of IL-6 is unknown, a number of reports indicate that myeloma cells produce factors that may be able to mediate this process. Reports have shown that certain myeloma cells can produce a number of cytokines and local growth factors, including IL-1β (Cozzolino *et al*., 1989; Carter *et al*., 1990), lymphotoxin (Garrett *et al*., 1987) and MCSF (Nakamura *et al*., 1989), which are able to induce the synthesis of IL-6 by hBMSCs and hBMSC lines. In addition, Ishimi *et al*. (1990) have shown that a number of factors, including IL-1β and tumour necrosis factor alpha (TNFα), can induce IL-6 mRNA expression in the MC3T3-E1 mouse osteoblastic cell line and primary osteoblast-like cells derived from fetal mouse calvaria. Furthermore, Linkhart *et al*. (1991) have demonstrated that IL-1β could increase IL-6 mRNA and protein production by human osteoblasts. Although these studies implicate a number of molecules in this activity, further studies will be required to determine the identity of the soluble factor(s) released by myeloma cells that may be responsible for mediating this response.

In this study, hOBs constitutively produced more IL-6 than hBMSCs isolated from the same donor. In addition, myeloma cells stimulated a significantly greater increase in the production of IL-6 from hOBs than from hBMSCs. The reasons for these differences remain unclear; however, as hBMSCs represent a heterogeneous population of cells, including cells of the osteogenic lineage, adipocytes and fibroblasts, it remains likely that their relative ability to produce IL-6 varies. In support of this, when hBMSCs were treated with dexamethasone, which has been shown to promote osteogenic differentiation, they were able to release larger amounts of IL-6. Furthermore, myeloma cells promoted the production of greater amounts of IL-6 from these hBMSCs than from untreated cells, suggesting that more well-differentiated osteogenic cells have a greater capacity to synthesize IL–6 after interaction with myeloma cells.

In conclusion, these studies have demonstrated that myeloma cells stimulate human osteoblasts to produce IL-6 and that this is mediated, in part, by both cell-to-cell contact and/or soluble factors. Furthermore, as hOBs produce more IL-6 than hBMSCs in this system and differentiation of hBMSCs towards cells with a mature osteoblast phenotype results in a greater ability to produce IL-6, it is likely that well-differentiated cells of the osteogenic lineage may play an important role in producing IL-6 in response to interactions with myeloma cells. This, in turn, may mediate the proliferation and survival of myeloma cells within the bone marrow microenvironment in a paracrine manner *in vivo.*

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